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Characterization of bacterial populations in Arctic permafrost soils using bacteriohopanepolyols

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Keywords

permafrost soils; active layer; microbial lipids; bacteriohopanepolyol (BHP)

Abstract

Bacteriohopanepolyols (BHPs) are biomarkers providing taxonomically and environmentally diagnostic information. BHPs may help to unravel bacterial communities residing in recent as well as ancient permafrost soils and sediments and also provide information on associated environmental conditions. However, detailed data on their distribution in the heterogeneous Arctic environment are scarce. The distribution and structural diversity of BHPs were studied in the annually thawing (active) layer of three different sites in the polygonal tundra of the Lena Delta in the Siberian Arctic. Variations between permafrost structures and soil horizons caused by differences in the physical and chemical soil properties were observed. C and N content significantly influences the BHP composition resulting in the highest BHP concentrations and greatest structural diversity in the uppermost organic soil horizons, which consist mainly of fresh or little degraded plant material. Furthermore, statistical analyses reveal that higher abundances of adenosylhopane-type soil-marker BHPs are linked to higher soil pH. Small-scale environmental controls on BHP distributions are reflected by amine-functionalized BHPs from methanotrophic bacteria only occurring in the water-saturated, oxygen-depleted polygon centres and by soil-marker BHPs, which are significantly more abundant in the well aerated polygon rims than in the centres. In contrast, C-2 methylated BHPs, putative indicators of plant-bacterial interactions, are present in all soil horizons and permafrost structures and their relative distribution is not systematically linked to soil properties. Overall, lipid-based results agree with published 16S rRNA based community structure assessments highlighting the usefulness of BHPs to represent bacterial populations in recent and ancient permafrost soils.

1. Introduction

Permafrost soils are a very distinct habitat for life given their sub-zero temperatures and significant temperature fluctuations (Gilichinsky et al., 1995). Nonetheless, microorganisms have adapted to these harsh conditions through metabolic regulation (Jansson and Tas, 2014 and reference therein). Bacterial activity and cell growth have been reported at ambient permafrost temperatures of down to -25°C (Mykytczuk et al., 2013). Generally, bacteria seem to occur in higher diversity and higher abundance when compared to both archaea and fungi (Steven et al., 2008, Yergeau et al., 2010). The most frequently observed bacterial phyla (based on 16S rRNA sequencing) are Proteobacteria, Firmicutes, Chloroflexi, Acidobacteria, Actinobacteria and Bacteroidetes (Jansson and Tas, 2014).

One means to study bacterial biomass is the analysis of their membrane lipids. Phospholipid fatty acids (PLFAs) are the main constituents of the bacterial bilayer membrane. However, PLFAs are degraded within days to weeks after cell death and, thus, reflect the viable microbial community (Kaur et al., 2005) and do not allow tracing of bacterial biomass through space and time. Bacteriohopanepolyols (BHPs) are pentacyclic triterpenoids, which are produced almost exclusively by bacteria, although not all bacteria, while they are absent in archaea (Ourisson and Albrecht, 1992). In contrast to PLFAs, the degradation products of BHPs can be preserved in sedimentary records (e.g., Brocks et al., 1999). BHPs are believed to help organisms adapt to physiological stress by modulating the fluidity and organization of the bacterial membrane (Rohmer et al., 1984; Kannenberg and Poralla, 1999; Rohmer, 2008; Welander et al., 2009; Sáenz et al., 2012). To date a suite of structurally diverse side chains have been identified of which some are proposed to provide taxonomic and/or physiological information (Rohmer, 1993; Talbot and Farrimond, 2007). For example, some amine-functionalized BHPs such as 35-aminobacteriohopane-31,32,33,34-tetrol (aminotetrol; **Ia**, see Appendix for structures) and 35-aminobacteriohopane-

30,31,32,33,34-pentol (aminopentol; **Ib**) are characteristic for aerobic methanotrophic bacteria and are found in many species and environments (Neunlist and Rohmer, 1985a; Cvejic et al., 2000; Talbot and Farrimond, 2007; Zhu et al., 2010; van Winden et al., 2012; Talbot et al., 2014). However, low levels of aminotetrol (**Ia**) and in one case trace levels of aminopentol (**Ib**) have been found in some species of sulphate-reducing bacteria of the genus *Desulfovibrio* (Blumenberg et al., 2006; Blumenberg et al., 2009; Blumenberg et al., 2012). BHPs methylated at the C-2 position (**II**) have previously been used as markers for cyanobacteria (e.g. Summons et al., 1999; Zhang et al., 2007; Talbot et al., 2008) although this interpretation has been questioned as the *hpnP* gene, required for C-2-methylation, has also be identified in Alphaproteobacteria and in at least one Acidobacterium (Welander et al., 2010; Ricci et al., 2014). The of C-2 methylation in Alphaproteobacteria appears to be particularly common in species involved in plant-microbe interactions and to be an indicator for this specific environmental niche rather than a taxonomic marker (Ricci et al., 2014).

The BHP adenosylhopane (**Ic**) has been reported to be the precursor to all other side chain extended BHPs (Bradley et al., 2010). Adenosylhopane (**Ic**) together with several related structures also containing a cyclised side chain (**Id, Ie**) and their C-2 methylated homologues (**IIc**, **IId**, **IIe**) are also potential environmental rather than taxonomic markers. These compounds have been identified to be abundant in soils while occurring only in minor concentrations in marine and lacustrine sediments (Talbot and Farrimond, 2007; Cooke et al., 2009; Xu et al., 2009; Blumenberg et al., 2010; Rethemeyer et al., 2010; Kim et al., 2011; Sáenz et al., 2011; Taylor and Harvey, 2011; Zhu et al., 2011; Doğrul Selver et al., 2012; Wagner et al., 2014). Adenosylhopane (**Ic**) and its related structures (hereafter referred to as soil-marker BHPs) have also been studied in Arctic environment where they have been used as indicators for the export of terrigenous organic matter into Arctic rivers and the Arctic Ocean (van Dongen et al., 2008; Cooke et al., 2009; Taylor and Harvey, 2011; Doğrul Selver et al., 2015). In the

Arctic studies mainly marine and riverine samples were investigated, while information from the alleged terrestrial source – permafrost soils – remains scarce. To date only two studies of BHPs in Arctic soils have been published. Rethemeyer et al. (2010) studied BHPs in Arctic permafrost soils from Svalbard in which a larger and more diverse bacterial community was observed in the organic horizons compared to the underlying mineral soils. Doğrul Selver et al. (2015) investigated three Yedoma (ice complex) samples within the Siberian Kolyma River catchment and found low structural BHP diversities dominated by soil marker BHPs. So far, no study has been performed in the organic-rich and extremely heterogeneous Arctic tundra even though these regions contribute significantly to the terrestrial organic matter pool (Ciais et al., 2013) – including BHPs – found in Arctic rivers and the Arctic Ocean (Dittmar and Kattner, 2003). Accordingly, understanding BHP distributions in these polygonal tundra soils is crucial for understanding the BHP records obtained from such Arctic riverine and marine samples off tundra areas. Here, we investigated BHP distributions in the seasonally thawing surface layer, the active layer, of characteristic polygonal tundra soils in the Siberian Arctic in order to (a) evaluate their spatial variability and (b) to characterize the bacterial communities present and compare these results with other methods including PLFA analyses and DNA/RNA sequencing.

2. Study area

The Lena Delta in Siberia, Russia, is the largest Arctic river delta covering approximately 32,000 km^2 (Are and Reimnitz, 2000). It is located in the zone of continuous permafrost under an Arctic continental climate characterised by low mean annual precipitation (125 mm), low mean annual air temperatures (-12.5°C), and a large seasonal temperature amplitude between summer (July 10.1°C) and winter (February -33.1°C; Boike et al., 2013).

The Lena Delta consists of over 1,000 islands including Samoylov Island and Kurungnakh Island (Fig. 1). Samoylov Island belongs to the recent active delta region (1-12 m a.s.l.) and is made up of Holocene fluvial sediment. It is characterised by ice wedge polygonal tundra with thermokarst lakes and an active flood plain. Ice wedges form in polygonal patterns through seasonal frost-cracking repeatedly pushing material upwards to form elevated rims surrounding depressed centres (e.g., Fiedler et al., 2004). Kurungnakh Island (30-60 m a.s.l.) consists of a lower, 15-20 m thick Paleo-Lena River sand unit overlain by a ca. 20 m thick late Pleistocene ice-rich, fine-grained permafrost sequence (ice complex; Schirrmeister et al., 2011). The ice complex formation, often synonymously called 'Yedoma', is covered by a 2-3 m thick unit of Holocene aeolian silty sand in which polygonal tundra developed with small, 3-5 m wide ice wedges (Morgenstern et al., 2011; Schirrmeister et al., 2011; Zubrzycki, 2013).

The soils on Samoylov and Kurungnakh Islands belong to the order of Gelisols (Soil Survey Staff, 2010) with polygon rims dominated by Glacic Aquiturbels and depressed polygon centres characterized by Typic or Ruptic Historthels with sandy loam to silt loam soil texture (Table 2; Zubrzycki et al., 2012). Mosses, grasses, sedges, and dwarf willow shrubs dominate the vegetation at both study sites with different distributions on polygon rims and centres (Boike et al., 2013).

3. Material and methods

3.1 Sampling

In total, three sampling sites were chosen according to their morphological differences (Table 1): a polygon in a relatively recently developed thermokarst basin (KUB) and a polygon on the elevated (55 m a.s.l) upland (KUU) on Kurungnakh Island as well as a polygon developed on typical modern fluvial deposits on Samoylov Island (SA). In comparison to the aeolian origin of the KUU polygon, the KUB polygon developed on

aeolian and lacustrine sediment of a former thermokarst lake, which drained about 5.7 ka BP (Morgenstern et al., 2013). At all sites, the polygons had diameters of ca. 9-13 m. Both the depressed, water saturated polygon centres (C) and the several decimetres elevated and relatively dry polygon rims (R) were sampled at the end of the summer, i.e. at maximal thaw depth of the active layer in August 2009 and 2010. Samples were taken from the characteristic horizons of the active layer (Table 2), which was about 29 to 43 cm thick; shallower in polygon rims and deeper in centres. Soil horizons were defined according to US Soil Taxonomy (Soil Survey Staff, 2010). At site SA, a sample of the uppermost still frozen permafrost was also retrieved from the polygon rim (SA-R_{Bjjf}).

All samples were stored in pre-combusted glass jars and kept frozen until analysis. Prior to analysis, samples were freeze-dried and ground.

3.3 Bulk soil analysis

Total carbon and nitrogen contents were determined on 5-10 mg soil using a Vario MICRO cube elemental analyser (Elementar, Germany). All samples were carbonate-free, therefore total C contents equal total organic carbon content (TOC). Soil pH values were measured in water (soil:water 1:2.5; w:w) one hour after water addition (IUSS Working Group WRB, 2006).

3.4 BHP extraction and analysis

BHPs were extracted using the method described in Cooke et al. (2008a). Briefly, samples (2 g) were sonicated 3 times using chloroform/methanol/water (5:10:4; v:v) followed by liquid-liquid extraction of the organics from the aqueous phase. Subsequently, an internal standard (5α–pregnane-3β,20β-diol) was added and the total lipid extract was acetylated using acetic anhydride and pyridine (1:1, v:v; heated at 50°C for one hour, left overnight), dried, and re-dissolved in methanol/propan-2-ol (3:2,

v:v) for filtration over 0.45 µm PTFE syringe filters prior to LC/MS^n measurements. Reversed-phase LC-MS^n analysis was performed using a Surveyor LC system (ThermoFinnigan, UK) equipped with an atmospheric pressure chemical ionization (APCI) ion source operated in positive ion mode. Separation of BHPs was achieved following the method and setup described by Talbot et al. (2007b). BHPs were identified according to their characteristic APCI base peak (m/z; Table 3) and quantified using the internal standard 5α–pregnane-3β,20β-diol (for details about the quantification procedure see van Winden et al., 2012). Only BHPs with good signal-to-noise ratios were quantified. The detection limit was 0.4 µg g^{-1} DW (dry weight). Additionally, an external standard was run six times (relative SD for the single BHPs: 3-11%) to ensure high quality of measurements. One sample (SA-C_{Oi}) was measured 3 times to check the analytical reproducibility (relative SD for the single BHPs: 5-42%).

3.5 Statistical analysis

In order to relate the BHP distributions to soil properties principal component analysis (PCA) was performed using PAST 3.06 for Windows (Hammer et al., 2001). Since the variables differ in their units, the PCA routine was run using the correlation matrix which implies normalizing all variables using division by their standard deviations (Hammer et al., 2001). Available data representing soil properties include C content, N content, soil pH texture (grain size), and water saturation. Unfortunately, in-situ soil temperature was not obtained for all soil profiles and is, thus, not included in the PCA. Variables determined in the field (i.e. texture and water saturation; Table 2) were converted into metric data. Grain size (soil texture) was calculated following the classification of the US Soil Taxonomy (Soil Survey Staff, 2010) using the maximum grain size of the soil texture (since a minimum is not defined for silt) and relative contribution of the sand, silt, and clay fractions and their mean fractional contributions to each texture class (Supplement Table S1). The grain size of the O horizons was

defined as 2 cm based on visible characteristics of the litter. Water saturation was defined as 100% (saturated), 70% (temporarily saturated), and 30% (not saturated/well aerated). These definitions are based on soil moisture measurements on Samoylov (Boike et al., 2008) since accurate water contents of the samples could not be determined due to excessive water leakage of saturated soil horizons during sampling.

4. Results

4.1 Bulk soil characteristics

TOC contents range from 2% to 28% between the different sites and soil horizons, generally decreasing with depth at all sites (Table 2). Soils on KU have higher TOC contents (from 4% to 28%) than soils on SA (2% to 23%). Between sites, TOC contents differ most significantly in the O horizons consisting of mainly non-degraded plant litter, which is reflected by high C/N ratios ranging from 19 to 69 (mean 39; n = 10). The KUB polygon has the highest TOC contents (28%) in the uppermost organic soil horizons (Oi) of all sites compared to significantly lower TOC contents in KUU (19-23%) and SA (ca. 10%). These differences may be due to the differences of the vegetation between sites, particularly between polygon rims (dominated by moss, grasses, shrubs) and polygon centres (dominated by moss and sedges). TOC contents are lower in the Oe horizons characterized by more strongly decomposed plant litter at all three sites with very similar values ranging from 7% to 11% (except in KUB-C with 23%) and C/N ratios of 19 to 51. At all sites the A and B horizons have lower TOC contents (2% to 16%) particularly at site SA-R (2% to 3%) and lower C/N ratios (16 to 35).

Soil pH values vary from pH 4.5 to 6.7 (Table 2). Except for the KUB polygon (KUB-R and KUB-C) where pH values are similar in all horizons. At all other locations pH values increase about 1 to 1.5 pH units with depth. Between sites, it is evident that pH values are slightly lower in the A and B horizons on KU (pH 4.5 to pH 6.3) than on SA (pH 4.8 to pH 6.7), which may be due to the different parent material at both sites.

4.2 Distribution of BHPs

Total BHP concentrations range from 84 to 1111 µg g^{-1} TOC showing different trends with increasing soil depth at the three sites (Fig. 2, Table 4). On SA BHP concentrations in the polygon rim and centre decrease with depth paralleling decreasing TOC values (from 523 to 286 µg g^{-1} TOC in the rim, and from 1111 to 84 µg g^{-1} TOC in the centre). On KU a moderate increase of BHP concentrations with soil depth is observed at KUB-C (from 107 to 244 µg g^{-1} TOC). In the other three soil profiles on KU a bimodal pattern of increasing and decreasing BHP concentrations is seen (KUB-R between 129 and 262 µg g^{-1} TOC; KUU-R between 105 and 333 µg g^{-1} TOC; KUU-C between 187 and 439 µg g^{-1} TOC). Differences of BHP concentrations are not associated with specific soil horizons. Only on SA the Oi horizons are characterised by the highest BHP concentrations (rim: 523 µg g^{-1} TOC and centre: 1111 µg g^{-1} TOC) while the Oi horizons on KU have the lowest BHP concentrations in the respective profiles (KUB-R: 129 µg g^{-1} TOC; KUB-C: 107 µg g^{-1} TOC; KUU-R; 105 µg g^{-1} TOC; KUU-C: 187 µg g^{-1} TOC).

In total, 17 different BHPs were identified (Table 4) in agreement with previously published results from the Arctic (Rethemeyer et al., 2010; Cooke et al., 2009). At all three sites the organic horizons (Oi and Oe) have higher structural diversity (10 to 15 BHPs) than the deeper A and B horizons, which consist of humified organic matter (4 to 10 BHPs; Fig. 3). Furthermore, the A and B horizons on KU have a higher structural diversity (4-10 BHPs) than on SA (6-8 BHPs). The most abundant BHPs in all samples are bacteriohopanetetrol (BHT; **If**), adenosylhopane (**Ic**), adenosylhopane-type-2 (**Id**), and BHT cyclitol ether (BHT-CE; **Ig**) accounting for 56-100% of the total BHP abundance similar to previous soil studies from various environmental locations (e.g., Cooke et al., 2008a; Rethemeyer et al., 2010; Kim et al., 2011; Zhu et al., 2011). Aminobacteriohopane-32,33,34-triol (aminotriol; **Ih**) and 2-Me BHT (**IIf**) were also

present at lower concentrations at all sites and depths except sample KUU-R_{Bg}. At all sites, the less abundant BHPs BHT pentose (**Ii**) and 2β-Me-bacteriohopane-32,33,34,35-tetrol pentose (2-Me BHT pentose; **IIi**), BHT glucosamine (**Ij**), bacteriohopane-31,32,33,34,35-pentol cyclitol ether (BHpentol-CE; **Ik**), bacteriohopane-30,31,32,33,34,35-hexol cyclitol ether (BHhexol-CE; **Im**), and a novel composite hexafunctionalised BHP (BHhexol-Comp; **In**) are almost exclusively found in the O horizons except for BHT glucosamine (**Ij**) in samples KUB-R_{Bjjg} and SA-C_B as well as BHpentol-CE (**Ik**) and BHhexol-CE (**Im**) in samples KUB-C_A and KUU-R_{Bjj} (Table 4, Fig. 3). Furthermore, aminotetrol (**Ia**) and aminopentol (**Ib**) are only present in the polygon centres, except aminopentol (**Ib**) in sample KUU-R_{Bjj}, and occur there almost only in the Oe and A horizons (except SA-C_B; Table 4, Fig. 3). Besides the frequently occurring adenosylhopane (**Ic**) and adenosylhopane-type-2 (**Id**) two more soil-marker BHPs are observed. The methylated structure 2-Me adenosylhopane-type-2 (**IId**) is only found in the KU samples and at low concentrations. In contrast, adenosylhopane-type-3 (**Ie**), which was first identified in active layer soils on Svalbard (Rethemeyer et al., 2010), occurs more often on SA than on KU, where it is present only in the Oe horizons (and one A horizon; KUU-R_A).

4.3 Principal component analysis

Running the PCA with all investigated soil horizons reveals that the first two principal components explain 55.8% of the total data variance (Supplementary Fig. S1). However, sample SA-C_{Oi} strongly defines the first principal component (PC1, 36.6%) – due to its high BHP structural diversity and absolute concentrations – while the soil properties are all represented by the second principal component (PC2, 19.2%). We, thus, also ran the PCA excluding sample SA-C_{Oi} (PCA-2; Fig. 4) to better assess the influence of soil properties on the BHP distributions.

For PCA-2 the first two principal components explain 43.7% of the total data variance with PC1 and PC2 accounting for 24.5% and 19.2%, respectively. C and N content as well as grain size are positively correlated with PC1, whereas pH is negatively correlated with PC1 and water saturation is negatively correlated with PC2 (Fig. 4; Supplementary Fig. S2 and S3). This results in a separation of the soil samples into three clusters: carbon and nutrient rich O horizons (at the upper end of PC1; negative PC2 scores), the KU mineral horizons and SA-C_B (at the lower end of PC2), and the SA mineral horizons (lowest end of PC1; positive PC2 scores).

5. Discussion

5.1 Spatial distribution of BHPs

Permafrost soils are often influenced by cryoturbation (Jones et al., 2010), which may result in an up- or downward transport of organic matter within the soil profile. However, at all sites distortion of soil horizons in the active layer was not obvious and ^{14}C results of bulk organic matter show a nearly linear increase in age with soil depth (Höfle et al., 2013 and unpublished data). We therefore assume that the spatial distribution of BHPs is not altered by cryoturbation but mirrors the in situ signal of the microbial community.

In general, the observed total BHP concentrations in the active layer on KU and SA (84-523 µg g^{-1} TOC; excluding sample SA-C_{Oi}, which has the extraordinarily high total BHP concentration of 1111 µg g^{-1} TOC) are in the range of previously reported results for the active layer near Ny-Ålesund, Svalbard (Rethemeyer et al., 2010: 18-661 µg g^{-1} TOC in 0-90 cm depth), but are higher than results for two Alaskan peat samples (40-85 µg g^{-1} TOC; Taylor and Harvey, 2011) and three Siberian Yedoma samples (127-338 µg g^{-1} TOC; Doğrul Selver et al., 2015). Similar to our results, Cooke et al. (2009) found BHP concentrations of 236-613 µg g^{-1} TOC in East Siberian shelf sediments including one sample off the Lena River, which had BHP concentrations of 289 µg g^{-1}

TOC. The surface sediment collected off the Lena Delta also had a similar structural diversity (12 BHPs; Cooke et al., 2009) to our samples (4 to 15 BHPs).

The observed BHP distributions vary spatially between sampling locations, i.e., the fluvial deposits on SA and aeolian deposits on KU, and with depth, i.e., in the different horizons of the active layer (Fig. 3, Tab. 4). These differences are likely due to a range of physical and chemical soil properties such as soil texture, moisture, temperature, pH, and redox conditions as well as sources and content of organic carbon, which all influence bacterial diversity and activity (Jansson and Tas, 2014; Landesman et al., 2014). For some of these environmental conditions including pH, temperature, and osmotic stress, adaptations of the BHP content of the bacterial cell membranes were shown under laboratory conditions (Poralla et al., 1984; Welander et al., 2009; Kulkarni et al., 2013; Wu et al., 2015). In order to determine those factors controlling BHP distributions and concentrations in our samples, we used PCA.

5.1.1 Influence of soil properties

The PCA-2 shows that the O horizons, which have a high BHP diversity due to the almost exclusive occurrence of a range of highly functionalised composite BHPs correlate positively with C and N content as well as grain size (Fig. 4). This is in line with the study on Svalbard where BHPs were more diverse in the organic horizons with high C contents compared to the mineral soils (Rethemeyer et al., 2010). The greater BHP structural diversity in the organic horizons may reflect greater bacterial diversity, which is in agreement with the spatial pattern observed in a 16S rRNA gene sequencing study on SA (Liebner et al., 2008). In this study the authors attributed the higher diversity to the higher availability of oxygen, nutrients, and dissolved organic matter in the near surface soil. The PCA-2 (Fig. 4) results show also that the distribution of BHT pentose (**Ii**), 2-Me BHT pentose (**IIi**), unsaturated BHT pentose (unsatBHT pentose: **IIIi** or **IVi**), BHT-glucosamine (**Ij**), BHpentol-CE (**Ik**), BHhexol-CE

(**Im**), and BHhexol-Comp (**In**) can be explained by PC1 and correlates positively with C and N content. BHpentol-CE (**Ik**), BHhexol-CE (**Im**), and BHhexol-Comp (**In**) also show an anti-correlation with water saturation (PC2) and pH (PC1). These composite BHPs could potentially be produced by bacteria specialised in aerobic organic matter (plant) degradation since heterotrophic bacteria should thrive under the observed carbon-rich conditions. Alternatively, the higher structural diversity in the O horizons could result from N-limitation since many BHP-producing bacteria can fix N_2 (e.g., Bravo et al., 2001; Rosa-Putra et al., 2001; Ricci et al., 2014) and could, thus, outcompete other bacteria in low N environments like Siberian permafrost regions (e.g., Sanders et al., 2010; Beermann et al., 2015). While the N contents of the O horizons are mostly higher than in the deeper horizons of the respective soil profile, we consider this N to be mostly bound in plant tissue (therefore not directly accessible to the bacteria) since both C contents and C/N ratios are extremely high. UnsatBHT pentose (**IIi** or **IVi**) abundances are not linked to any soil properties since it was only occurs in sample (KUU-R_{Oi}).

The PC2 also reveals a positive correlation between pH and soil-marker BHP abundances, specifically adenosylhopane (**Ic**), adenosylhopane-type-2 (**Id**), and -type-3 (**Ie**). Earlier studies demonstrated that hopanoids can minimize pH-induced changes in membrane fluidity and order (Poralla et al., 1984; Welander et al., 2009; Sáenz et al., 2012). The accumulation of adenosylhopane (**Ic**), which is the first intermediate in BHP production via side chain elongation (Bradley et al., 2010) in soils with higher pH values on SA could indicate that bacteria may not need to further extend the BHP side chains to adapt their cell membrane at relatively neutral pH. While more complex/composite side chains may be synthesized to maintain membrane order/fluidity at lower soil pH (e.g. Poralla et al., 1984). Potentially, this pH response is characteristic for the location investigated here and in other cold environments where accumulation of soil-marker BHPs is particularly high (e.g. Canada, Svalbard, and Siberia; Xu et al., 2009;

Rethemeyer et al., 2010; Doğrul Selver et al., 2015). In comparison, Kim et al. (2011) found lower soil-marker BHP concentrations in soils with higher pH in temperate France albeit these authors also found higher BHP structural diversities at lower soil pH. Thus, BHP side chain elongation in response to pH adaption might be further controlled by climatic conditions.

Recently, Ricci et al. (2014) demonstrated that C-2 methylation of BHPs might be particularly common in bacterial species associated with plants and in suboxic and anoxic environments with low N and high osmolarity. Some of these environmental features are also prominent in our samples and should be related to the observed 2-Me BHP abundances albeit only three 2-Me BHPs could be detected. PCA-2 shows that the 2-Me BHP abundances in our samples are not systematic (Fig. 4). 2-Me adenosylhopane-type-2 (**IId**) abundances are not correlated with the first two principal components (Supplementary Fig. S2 and S3) thus, with none of the investigated soil properties. 2-Me BHT (**IIf**) abundances are explained by PC 2 and show an anti-correlation with water saturation implying 2-Me BHT (**IIf**) is linked to the better-aerated soil horizons. 2-Me BHT pentose (**IIi**) abundances correlate with C and N contents as well as water saturation due to its exclusive occurrence in the O horizons of the water-saturated polygon centres similar to BHT glucosamine (**Ij**) and aminotetrol (**Ia**) and aminopentol (**Ib**). The restriction of aminotetrol (**Ia**) and aminopentol (**Ib**) to the polygon centres (except SA-C_B) is consistent with high methane oxidation rates in these horizons as observed by Wagner et al. (2005). Both BHPs have so far only been identified in methanotrophic bacteria (Neunlist and Rohmer, 1985c; Neunlist and Rohmer, 1985a; Cvejic et al., 2000; Coolen et al., 2008; Talbot et al., 2008; van Winden et al., 2012) and a few species of the strictly anoxic sulphate-reducing genus *Desulfovibrio* (Blumenberg et al., 2006; Blumenberg et al., 2012). Accordingly, we consider aminotetrol (**Ia**) and aminopentol (**Ib**) in the polygon centres indicative for bacteria adapted to the oxygen-depleted conditions. The differentiation between rims

and polygon centres is consistent with 16S rRNA gene sequencing results of Liebner et al. (2008) who found polygon centres and rims on SA were dominated by different bacteria. Also, the 16S rRNA data of Wagner et al. (2009) indicate both different bacterial communities and activities between the anaerobic and aerobic parts of a polygon centre on SA. In both studies, the redox conditions were considered the most important factor controlling the bacterial community structure.

We consider the observed spatial distribution of BHPs to truly represent the spatial distribution of different bacterial communities or different environmental niches. The decrease of the BHP structural diversity with depth (Fig. 2) and occurrence of many highly functionalised composite BHPs only in the uppermost horizons (Table 4) could in fact also simply be a function of BHP preservation. In such a scenario, low complexity-BHPs (i.e. non-composite structures) could represent degradation products of more complex BHPs such as BHT pentose (**Ii**), BHpentol-CE (**Ik**), BHhexol-CE (**Im**), and BHhexol-Comp (**In**). Indeed, BHT (**If**) has been shown to be a degradation product of BHT glucosamine (**Ij**) during simulated diagenetic conditions (Schaeffer et al., 2010), which is also reflected by the anti-correlation of these BHPs observed in the PCA-2 results (Fig. 4). At lower pH preservation effects could even be amplified by decreasing microbial activities at lower pH (van Bergen et al., 1998; Nierop et al., 2005). However, several other BHPs including all observed soil-marker BHPs and BHT glucosamine (**Ij**) show increasing absolute concentrations with depth at all sites (Table 4) and BHP abundances do not show a negative correlation with pH. Increased concentration of soil-marker BHPs with depth is unexpected in comparison to analysis of deep-sea fan sediments in which adenosylhopane was found to be the most labile BHP compared to other compounds including both composite and non-composite structures (Cooke et al., 2008b; Handley et al., 2010). Thus, the observed pattern more likely reflects different bacterial communities or physiological adaptations in the different soil horizons.

5.2 Assessment of BHP-producing bacterial communities

In the following section, we provide an assessment of the BHP-producing bacterial community structure found in the active layer of the polygonal tundra aiming to extend the limited taxonomic information carried by BHPs through comparison with published genomic data (Table 5) as well as other lipid data from the study area. This adds to understanding the information carried by BHPs in ancient permafrost deposits which do not contain DNA traces. Table 5 summarizes bacterial species known to produce BHPs or possess genes encoded in BHP synthesis (*sqhC, hpnP*) to date listed against 16S rRNA sequencing data of Samoylov soils from earlier studies (Liebner, 2007; Liebner et al., 2008). While DNA and RNA are typically prone to rather quick degradation and, thus, represent viable communities, BHPs assemblages might also include fossil components (e.g. Talbot et al., 2014). However, since permafrost soils persist at sub-zero temperatures they are ideal settings for the preservation of DNA and RNA (Willerslev et al., 2004a; Willerslev et al., 2004b). Willerslev et al. (2004b) showed that bacterial rDNA could be reproducibly amplified from Siberian and Antarctic permafrost samples up to 400 kyr. Accordingly, both BHP and rRNA data obtained from Lena delta soils likely derive from living bacteria as well as dead and dormant cells.

5.2.1 Methanotrophic bacteria

As discussed above (section 5.1.2), the polygon centres and rims are defined by different redox conditions, which is reflected also in higher methane production and oxidation rates in the centres (Wagner et al., 2005). Given the high methane concentrations and the fact that the polygon centres are not fully anoxic (Zubrzycki, 2013), we exclude *Desulfovibrio sp*. as a source of aminotetrol (**Ia**) and aminopentol (**Ib**) and consider methanotrophic bacteria the most likely source organisms (Talbot et al., 2014).

Methanotrophs use two different ways to assimilate carbon; type I methanotrophs use the ribulose monophosphate (RuMP) pathway, while type II methanotrophs use the serine pathway (Hanson and Hanson, 1996). While aminotetrol (**Ia**) is produced by both type I and type II methanotrophs, aminopentol (**Ib**) seems to be a marker for most type I methanotrophs (Neunlist and Rohmer, 1985c; Talbot et al., 2001; van Winden et al., 2012). In the samples of this study, aminotetrol (**Ia**) and aminopentol (**Ib**) were detected in similar quantities (Table 4), therefore, type I methanotrophs must be the dominant source of both amino-BHPs. This is in agreement with previous studies on SA identifying type I methanotrophs dominant over type II methanotrophs using PLFA-based approaches (Wagner et al., 2005; Knoblauch et al., 2008).

Wagner et al. (2005) and Liebner et al. (2009) determined higher cell counts of methanotrophic bacteria in the polygon rims on SA than in the corresponding centres. We only detected aminopentol (**Ib**) in one polygon rim sample (KUU-R_{Bjj}) and no aminotetrol (**Ia**) in any rim suggesting that the concentration might be too low to be detected or both BHPs are readily oxidised under the more oxygenic conditions in the rims.

Among the type I methanotrophs Liebner (2007) only identified *Methylobacter psychrophilus* (Methylococcaceae) in a polygon rim on SA, which has not yet been identified as BHP-producer. However, several genera of the Methylococcaceae (type I methanotrophs) produce aminotetrol (**Ia**) and/or aminopentol (**Ib**) or possess the squalene-hopene cyclase gene (*sqhC*) required for BHP production (Table 5). Aminotetrol (**Ia**) and aminopentol (**Ib**) in the active layers on SA and KU either indicate the presence of any of these type I methanotrophic genera not resolved by 16S rRNA (Liebner, 2007) or *Methylobacter psychrophilus* could be a yet unknown producer of these amino-BHPs. The absence of C-3 methylated aminopentol, abundant in various methanotrophic genera like *Methylococcus and Methylocaldum* (Cvejic et al., 2000),

makes it unlikely that species of these two genera are present in the studied active layers.

5.2.2 Soil-marker BHP-producing bacteria

Amongst those four BHPs regarded the most specific soil-markers adenosylhopane (**Ic**) and adenosylhopane-type-2 (**Id**) have previously been identified in various Alphaproteobacteria species, one betaproteobacterial and one cyanobacterial species (Table 5). In contrast, 2-Me adenosylhopane-type-2 (**IId**) and adenosylhopane-type-3 (**Ie**) have not yet been identified in any cultured organism. Using 16S rRNA sequencing, Liebner et al. (2008) found that Bacteriodetes, Actinobacteria, and Thermomicrobia dominate the soil bacterial communities in similar polygon soils on SA, none of which have been shown to produce BHPs albeit some Actinobacterial species possess the *sqhC* gene (Table 5). Liebner et al. (2008) and Liebner (2007) also identified several Alpha- and Betaproteobacteria including the family Bradyrhizobiaceae and the order Nitrosomonadales, respectively, but could not identify these beyond the family or order level. Adenosylhopane (**Ic**) and adenosylhopane-type-2 (**Id**) in our active layer profiles may therefore indicate the presence of Bradyrhizobiaceae and/or Nitrosomonadaceae, as they have previously been identified in *Bradyrhizobium japonicum* and *Rhodopseudomonas palustris* (both Bradyrhizobiaceae) as well as *Nitrosomonas europaea* (Nitrosomonadales). Additionally, adenosylhopane (**Ic**) and related compounds could be produced other related species of the Bradyrhizobiaceae and/or Nitrosomonadaceae, which have so far been identified to carry the *sqhC* gene (Table 5). These species could therefore also be putative BHP-producers for the yet unassigned 2-Me adenosylhopane-type 2 (**IId**) and adenosylhopane-type-3 (**Ie**). Since *B. japonicum* is the only species known so far to produce both methylated and non-methylated adenosylhopane-type-BHPs (Talbot et al., 2007b), anti-correlation of 2-Me adenosylhopane-type-2 (**IId**) with the

other adenosylhopane-type BHPs might simply be an adaptation of a Bradyrhizobium to variable environmental stress.

5.2.3 Putative plant-associated BHP-producing bacteria

C-2 methylated BHPs have often been regarded as indicative for cyanobacteria since they were observed in many cyanobacterial mats and cultures (Summons et al., 1999; Jahnke et al., 2004; Zhang et al., 2007; Talbot et al., 2008; Doughty et al., 2009). However, recently Ricci et al. (2014) evaluated the diversity of the *hpnP* gene, required for C-2 methylation, in the environment and found it particularly common in species forming commensal or mutualistic symbiotic interactions with plants and in environments low in oxygen and N and but with high osmolarity. Thus, 2-Me BHPs are putative indicators for this specific environmental niche. *HpnP* and/or C-2 methylated BHPs have been identified in various Alphaproteobacteria especially within the Bradyrhizobiaceae (Vilcheze et al., 1994; Bravo et al., 2001; Rashby et al., 2007; Welander et al., 2009; Welander et al., 2010; Blumenberg et al., 2013; Ricci et al., 2014).

Plant and microbial communities interact in various ways in Arctic soils (reviewed in Bell et al., 2013), especially many Alphaproteobacteria, in particular Bradhyrhizobiceae and Cyanobacteria are known to interact with plants (Ricci et al., 2014) and were also identified on SA by Lieber (2007) and Liebner et al. (2008; Table 5). Particularly the Bradhyrhizobiceae might, thus, be putative producers of 2-Me BHPs in our samples.

5.2.4 Unknown BHP-producing bacteria

Most information about BHP production comes from culture studies (see Talbot et al., 2008 for review), however, >99% of all bacterial species are not cultivatable (Epstein, 2013). Metagenomics and bioinformatics studies indicate that sequences from BHP-producers in environmental samples agree less than 60% (at the amino acid level) with

their relative BHP-producers in pure cultures (Pearson et al., 2007; Pearson and Rusch, 2009). Accordingly, the vast majority of BHP-producing bacterial species remain unidentified.

Unassigned BHPs in our samples include 2-Me adenosylhopane-type-2 (**IId**), adenosylhopane-type-3 (**Id**), BHhexol-CE (**Im**), and BHhexol-Comp (**In**) although a structurally similar BHhexol-manosamine has been reported from a species of *Alicyclobacillus* (Řezanka et al., 2011). As stated above, species of the families Bradyrhizobiaceae and Nitrosomonadaceae could be producers of the 2-Me adenosylhopane-type-2 (**IId**) and of the adenosylhopane-type-3 (**Id**).

Both unassigned composite BHhexols (**Im**, **In**) only occur in the O horizons on SA and KU and amongst these with higher abundance in the well-aerated rims. Liebner (2007) identified several *Geobacter* species and unaffiliated genera including *Syntrophus*, *Planctomyces*, Gemmata, Pirellula, and *Rubrobacter* in the active layers of a polygon on SA (Table 5). Within the genus *Geobacter*, two species are known to produce BHPs (Table 5). However, Liebner (2007) only detected *Geobacter* in the deeper, more anaerobic soil horizons of the polygon rim and in the anaerobic centres. Furthermore, guanidine-substituted BHT cyclitol, a major BHP product of the two *Geobacter* species (Fischer et al., 2005; Eickhoff et al., 2013), is not detected in any of our samples. Therefore, it is most unlikely that species of the genera *Geobactera* are potential BHP-producers of the composite BHhexols in the active layer profiles investigated. Several species of the bacterial genera found in the upper soils on SA have been recognised to possess the *sqhC* gene (Table 5) and particularly species of the genus *Syntrophus* and/or the phylum *Planctomycetes*, may be potential producers of BHhexol-CE (**Im**) and BHhexol-Comp (**In**).

6. Conclusion

BHP distributions differ between soil horizons and between permafrost structures (polygon rim and centre) of the three investigated active layer profiles in the Siberian Lena Delta. Statistical analysis reveals that the different soil properties, most importantly water saturation level, C and N content, and pH, influence the BHP-producing microbial community. Biomarkers for methanotrophic bacteria are almost exclusively present in polygon centres, which seems indicative of bacteria adapted to water-saturated and oxygen-depleted conditions. C and N contents are major soil properties influencing the relative distribution and structural diversity of the BHPs which is highest in the upper C- and N-rich O horizons due to the almost exclusive occurrence of several highly functionalised BHPs. This probably reflects a more diverse microbial community in the uppermost O soil horizons. Furthermore, soil pH governs BHP abundances. Higher soil pH is positively correlated with adenosylhopane-type soil-marker BHPs, which are not C-2 methylated. This might indicate the need for further side chain adaptation at lower pH while adenosylhopane-type BHPs may be sufficient to maintain membrane fluidity at more neutral pH under the prevalent climatic conditions. Finally, the occurrence of 2 Me BHPs cannot be unanimously linked to any of the investigated environmental parameters, but the distribution of each of the 2 Me BHPs is determined by different factors.

The BHP distribution in the different soil horizons agrees well with previously published bacterial community assessments based on 16S rRNA and PLFA analyses. Overall, the good agreement of the BHP data with 16S rRNA and PLFA-based analyses on SA suggests that BHPs are promising biomarkers to provide taxonomic and environmental information in recent Arctic permafrost. Therefore, BHP biomarkers provide a good tool to study bacterial biomass and communities in ancient permafrost.

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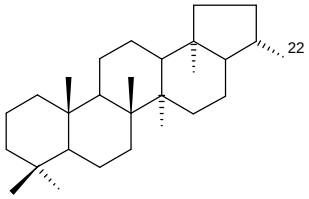
APPENDIX

Ring system and side chains of BHPs observed in Lena Delta samples. Side chain structures **d**, **e**, **i**, **m** and **n** are based on LC-MS^n analysis only. All other side chain structures shown have previously been unambiguously identified by NMR. When identified in this study using LC-MS^n only where stereochemistry cannot be confirmed, we have assumed the structure to be the same as that previously characterised but the occurrence of additional/alternative isomers cannot be excluded. Structures **d** and **e** differ in the mass of the terminal group (R) and are distinguished by LC-MS^n based on protonated molecular ions (see Table 3).



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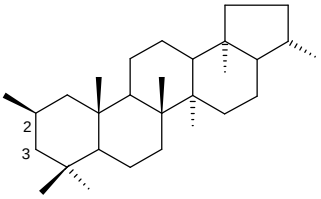

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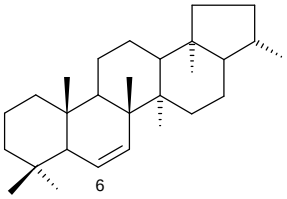

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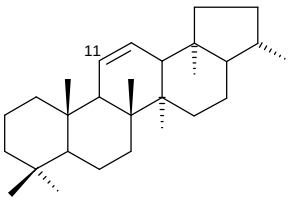

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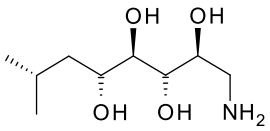
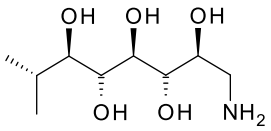


a



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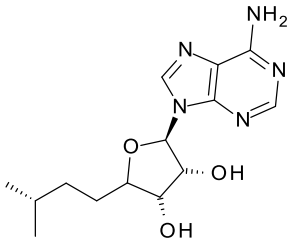

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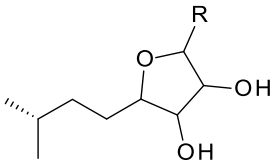
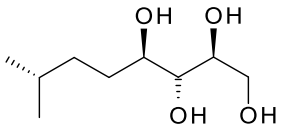


d or **e**

(R = unknown)



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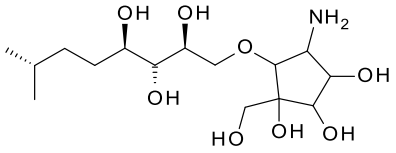

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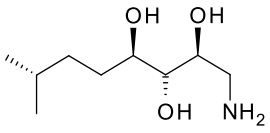

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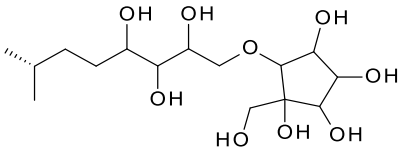

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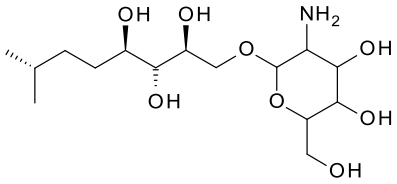

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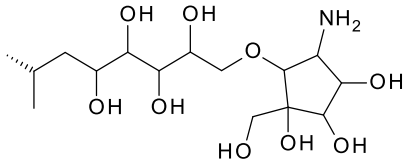

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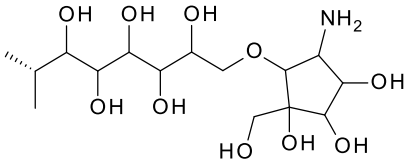

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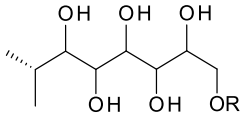


n (R= unknown)

Tables

Table 1: Study sites in the Lena Delta, Siberia (Russia).

Site	Latitude (N)	Longitude (E)	Elevation [m a.s.l.]
Samoylov Island (SA)	72° 22' 22''	126° 29' 05''	12
Kurungnakh Island – Thermokarst basin (KUB)	72° 19' 24''	126° 13' 22''	38
Kurungnakh Island – Upland (KUU)	72° 19' 31''	126° 15' 56''	55

Table 2: Basic soil properties.

Site location	Soil type – Great Group[a]	Horizon[a]	Depth [cm]	Texture[a]	Water saturated	pH	TOC[b] [%]	Total N [%]	C/N
Kurungnakh Island									
Thermokarst basin-	Glacic	Oi	0-12	litter	no	5.0	22.7	0.5	47
polygon rim	Aquiturbels	Oe	12-18	litter	no	5.0	6.9	0.4	19
(KUB-R)		Bjjg	18-30	loam	temporary	4.9	4.2	0.2	19
Thermokarst basin-	Ruptic	Oi	0-5	litter	yes	6.3	28.4	0.8	35
polygon centre	Historthels	Oe	5-12	litter	yes	4.9	23.0	0.7	33
(KUB-C)		Oe2	12-20	litter	yes	4.6	10.7	0.4	29
		A	20-28	silt loam	yes	4.7	15.7	0.7	23
		Bg	28-45	loamy sand	yes	5.2	5.6	0.3	22
Upland-	Glacic	Oi	0-10	litter	no	4.4	19.1	0.3	69
polygon rim	Aquiturbels	A	10-14	sandy loam	no	5.9	8.4	0.4	19
(KUU-R)		Bjj	14-16	sandy loam	no	5.3	8.4	0.5	16
		Bg	16-29	sandy loam	temporary	5.3	5.0	0.2	22
Upland-	Typic	Oi	0-6	litter	yes	5.2	21.1	0.6	35
polygon centre	Historthels	Oe	6-22	litter	yes	4.5	9.3	0.4	23
(KUU-C)		A	22-33	silt loam	yes	4.7	11.2	0.6	18
		Bg	33-48	sandy loam	yes	4.8	3.8	0.1	31
Samoylov Island									
Samoylov-	Glacic	Oi	0-7	litter	no	5.0	10.3	0.3	35
polygon rim	Aquiturbels	Ajj	7-13	sandy loam	no	6.1[c]	3.0[c]	0.2	19[c]
(SA-R)		Bjjg	13-18	loamy sand	no	6.4[c]	1.5[c]	0.1	18[c]
		Bjjg2	18-32	silt loam	temporary	6.3[c]	3.0[c]	0.2	18[c]
		Bjjf	32-37	silt loam	temporary	6.7[c]	2.5[c]	0.2	16[c]
Samoylov-	Typic	Oi	0-14	litter	yes	5.0	23.0	0.5	45
polygon centre	Historthels	Oe	14-40	litter	yes	4.8	8.7	0.2	51
(SA-C)		B	40-43	sandy loam	yes	5.9	4.2	0.2	35

[a] Classification, soil horizons and texture designations according to US Soil Taxonomy (Soil Survey Staff, 2010)
[b] total organic carbon
[c] Höfle et al. 2013 (depth of Ajj set to zero in the paper)

Table 3: List of identified BHPs according to BHP group including trivial name, structure, characteristic APCI base peak ion (m/z), and known source organisms.

BHP group	BHP trivial name and structure number	Base peak (m/z)	Source organisms	Supporting reference
Putative plant-associated	2-Me BHT (**IIf**)	669	Cyanobacteria + PNSB[a] + Alphaproteobacteria	Bisseret et al., 1985; Rashby et al., 2007; Welander et al., 2010; Ricci et al., 2014
	2-Me BHT pentose (**IIi**)	957	Cyanobacteria + Alphaproteobacteria	Talbot et al., 2008; Ricci et al., 2014
Methanotrophs	Aminotetrol (**Ia**)	772	Methanotrophs + sulfate reducing bacteria	Neunlist and Rohmer, 1985a; Blumenberg et al., 2006
	Aminopentol (**Ib**)	830	Methanotrophs type 1	Neunlist and Rohmer, 1985b
Soil-marker	Adenosylhopane (**Ic**)	746	PNSB + N_2 fixing + *Nitrosomonas europaea* + *Methylocella palustris*	Neunlist and Rohmer, 1985c; Bravo et al., 2001; Seemann et al., 1999; Talbot et al., 2007b; van Winden et al., 2012
	Adenosylhopane-type-2 (**Id**)	761	PNSB	Talbot et al., 2007b
	2-Me adenosylhopane-type-2 (**IId**)	775	Unknown	Cooke et al., 2008
	Adenosylhopane-type-3 (**Ie**)	802	Unknown	Rethemeyer et al., 2010
Various sources	Bacteriohopanetetrol (BHT) (**If**)	655	Cyanobacteria + PNSB + N_2 fixing + other sources	Bisseret et al., 1985; Neunlist and Rohmer, 1985b; Neunlist et al., 1988; Cvejic et al., 2000; Blumenberg et al., 2006; Talbot et al., 2008
	Amino triol (**Ih**)	714	Cyanobacteria + PNSB + N_2 fixing + methanotrophs + other sources	Neunlist and Rohmer, 1985b; Flesch and Rohmer, 1988; Neunlist et al., 1988; Seemann et al., 1999; Bravo et al., 2001; Blumenberg et al., 2006; Talbot et al., 2008
	unsaturated BHT pentose (unsatBHT pentose) (**III** or **IVi**)	941	Cyanobacteria (enrichment culture)	Talbot et al., 2008
	BHT pentose (**Ii**)	943	Cyanobacteria (enrichment culture)	Talbot et al., 2008
	BHT cyclitol ether (BHT-CE) (**Ig**)	1002	Cyanobacteria + PNSB + N_2 fixing + other sources	Renoux and Rohmer, 1985; Neunlist et al., 1988; Talbot et al., 2003; Joyeux et al., 2004; Talbot et al., 2007b; Talbot et al., 2008
	BHT glucosamine (**Ij**)	1002	Cyanobacteria + PNSB + N_2 fixing + other sources	Renoux and Rohmer, 1985; Talbot et al., 2007b
	BHpentol-CE (**Ik**)	1060	Cyanobacteria + N_2 fixing + other sources	Talbot et al., 2003; Joyeux et al., 2004; Talbot et al., 2007a
Unknown sources	BHhexol-CE (**Im**)	1118	Unknown	Talbot and Farrimond, 2007
	Bhhexol-Comp (**In**)	1132	Unknown	Cooke, 2011

[a] PNSB – purple non-sulfur bacteria

Table 4: BHP concentrations [µg g^{-1} TOC] of Kurungnakh Island (KU) and Samoylov Island (SA) soils. The compound structure number is given followed by base peak m/z.

Site location	Depth [cm]	Horizon	Number of BHPs	Putative plant-associated		Soil markers				Methanotrophs		Various sources							Unknown		Total BHPs
				IIf - 669	IIi - 957	Ic - 746	Id - 761	IId - 775	Ie - 802	Ia - 772	Ib - 830	If - 655	Ih - 714	IIIi or IVi - 941	Ii - 943	Ig - 1002	Ij - 1002	Ik - 1060	Im - 1118	In - 1132	
Kurungnakh Island																					
Thermokarst basin-	0-12	Oi	12	6		7	9	2				41	5		20	24	2	2	7	4	129
polygon rim	12-18	Oe	10	13		43	38	7	11			65	5			74		4	2		262
(KUB-R)	18-30	Bjjg	8	10		14	29	7				56	5			49	2				171
Thermokarst basin-	0-5	Oi	11	8	9	3	8					37	1		12	26		1	2	1	107
polygon centre	5-12	Oe	13	11		6	11	1		1	1	42	3			64	1	1	1	1	144
(KUB-C)	12-20	Oe2	12	8		8	10	2	3	1	1	49	6			62		1	1		152
	20-28	A	10	12		10	17	4		1		56	4			92		1	1		198
	28-45	Bg	6	10		11	17					127	9			69					244
Upland -	0-10	Oi	13	6		8	10	2				25	3	13	10	16	1	2	2	7	105
polygon rim	10-14	A	8	14		14	36	4	5			60	3			24					160
(KUU-R)	14-16	Bjj	10	24		28	59	13			1	74	10			117		3	3		333
	16-29	Bg	6	10		23	35					108	8			55					240
Upland -	0-6	Oi	13	10	10	20	27	1				46	1		19	32	1	7	5	5	187
polygon centre	6-22	Oe	12	29		22	47	4	2		2	110	12			201		3	4	3	439
(KUU-C)	22-33	A	9	28		16	36	6		3	2	149	16			155					411
	33-48	Bg	4			17	26					157				67					267
Samoylov Island																					
Samoylov -	0-7	Oi	10	30		47	20					114	20		26	135		20	44	67	523
polygon rim	7-13	Ajj	7	9		52	112		9			45	5			12					245
(SA-R)	13-18	Bjjg	7	28		28	212		12			127	8			21					436
	18-32	Bjjg2	7	10		81	126		18			108	19			65					427
	32-37	Bjjf	6			68	83		16			81	16			23					286
Samoylov -	0-14	Oi	15	34	88	59	106		4	11	8	201	45		108	383	16	25	14	10	1111
polygon centre	14-40	Oe	12	3	7	11	7			5	2	51	37		7	76	3	1			211
(SA-C)	40-43	B	8	3		4	6			2		32	14			20	2				84

Table 5: Overview of bacteria known to produce BHPs or/and possessing the *sqhC* or *hpnP* genes required for BHP or 2-Me BHP synthesis, respectively, as well as 16S rRNA revealed occurrences in soils on Samoylov Island based on published literature.

Phylogenetic affiliation	Species	BHP	Genes	Samoylov 16S rRNA [27, 28]
Acidobacteria				
Acidobacteriaceae	*Acidobacteria bacterium*		*sqhC* [23], *hpnP* [24]	
Solibacteraceae	*Solibacter usitatus*		*sqhC* [23], *hpnP* [24]	
unaffiliated				R_t, R_b, C_t, C_b
Actinobacteria				
Cellulomonadaceae	*Cellulomonas* sp.			R_b, C_b
	Oerskovia sp.			R_b
Frankiaceae	*Frankia* sp.	If [1]	*sqhC* [23, 25]	
	Frankia alni		*sqhC* [23, 25]	
Intrasporangiaceae	unaffiliated *Intrasporangiaceae*			R_b, C_t
Microbacteriaceae	*Cryobacterium sp.*			R_t
Nocardiaceae	*Rhodococcus sp.*			R_t
Propionibacteriaceae	*Propionibacterium sp.*			R_b, C_t, C_b
Pseudonocardiaceae	Pseudonocardia sp.			R_t
Rubrobacteraceae	*Rubrobacter xylanophilus?*		*sqhC* [23]	
	Rubrobacter sp.			R_b, C_t
Conexibacteraceae	*Conexibacter* sp.			R_t, C_b
unaffiliated				R_t, R_b, C_t, C_b
Bacteroidetes-Chlorobi				
Bacteriodaceae	*Bacteroides* sp.			R_t, R_b, C_t
	Bacteroides distasonis			R_b
Sphingobacteriaceae	*Flexibacter canadensis*			R_t, R_b
	Unaffiliated			R_t, R_b, C_t, C_b
Flavobacteriaceae	Flavobacterium ferrugineum			R_t
	Unaffiliated			R_t
unaffiliated Bacteriodetes				R_t
unaffiliated Chlorobi				R_t, R_b, C_t, C_b
Cyanobacteria				
Chroococcales (order)	*Acaryochloris marina*		*sqhC* [23]	
	Crocosphaera watsonii	If +Ig [2]	*sqhC* [23, 25]	
	Synechocystis sp.		*sqhC* [23, 25]	
	Thermosynechococcus elongatus		*sqhC* [23, 25], *hpnP* [24]	
Cyanobacteriaceae	*Cyanothece* sp.	If + IIf [2]	*sqhC* [23], *hpnP* [24]	
Microcystaceae	*Gloecocapsa* sp.	Ig + Ii + IIi + If + IIf [2]		
	Microcystis aeruginosa	Ih [2]	*sqhC* [23]	
Gloeobacteraceae	*Gloeobacter violaceus*		*sqhC* [23], *hpnP* [24]	
Chlorogloeopsidaceae	*Chlorogloeopsis fritschii*	If + IIf [2]		
Nostocaceae	*Anabena* sp.		*sqhC* [23]	
	Anabena variabilis		*sqhC* [23, 25]	
	Nostoc muscorum	If + IIf [3]		
	Nostoc punctiforme	If + IIf [4]	*sqhC* [23], *hpnP* [24]	
	Nostoc sp.	If + IIf [5]	*sqhC* [23], *hpnP* [24]	
Rivulariaceae	*Calothrix* sp.	IIf [2]		
Phormidiaceae	*Phormidium luridum*	If + IIf [2]	*hpnP* [26]	
Oscillatoriales (order)	*Trichodesmium erythraeum*	Ig [2]	*sqhC* [23, 25]	
Xenoccaceae	*Chroococcidiopsis* sp.	Ic + IIf [2]		
Prochlorotrichaceae	*Prochlorothrix hollandica*	If + IIf [2]		
?	*Oscillatoriales cyanobacterium*		*hpnP* [26]	
?	*Cyanobacterium aponium*		*hpnP* [26]	
?	*Microcoleus* sp.		*hpnP* [26]	
?	*Synechococcus* sp.		*hpnP* [26]	
unaffiliated				R_t
Firmictures				
Bacillaceae	*Bacillus macroides*			R_t
Clostridiaceae	*Clostridium*			R_b, C_t, C_b

Lachnospiraceae	*Johnsonella* sp.			R_b
Ruminococcaceae	*Acetivibrio* sp.			R_b, C_b
Acidaminococcaceae	*Sporotalea* sp.			C_b
Veillonellaceae	*Propionispira* sp.			R_b, C_t, C_b
unaffiliated				C_b
Gemmatimonadetes				
unaffiliated Gemmatimonadales				R_t
Planctomycetes				
Brocadiaceae	*Candidatus Kuenenia sp.*			R_t, C_t
	Candidatus Kuenenia stuttgartiensis	If + Ig [6]	*sqhC* [23, 25]	
Planctomycetaceae	*Gemmata* sp.			R_t
	Gemmata obscuriglobus		*sqhC* [23]	
	Pirellula sp.			R_t, C_t
	Planctomyces sp.			R_t
	Planctomyces maris		*sqhC* [23]	
Proteobacteria				
Alphaproteobacteria				
Beijerinchiaceae	*Beijerinckia indica* subsp. *indica*	If + Ih [7]	*hpnP* [24]	
	Methylocella palustris	Ia + Ic + If [8]		
	Methylocella silvestris		*hpnP* [24]	
Bradyrhizobiaceae	*Afipia bromeae*		*hpnP* [26]	
	Afipia clevelandersis		*hpnP* [26]	
	Afipia felis		*hpnP* [26]	
	Afipia sp.		*hpnP* [26]	
	Bradyrhizobiaceae bacterium		*hpnP* [26]	
	Bradyrhizobium japonicum	Ic + Ih [9]	*sqhC* [23], *hpnP* [24]	
	Bradyrhizobium sp.		*sqhC* [23], *hpnP* [24]	
	Nitrobacter hamburgensis		*sqhC* [23], *hpnP* [24]	
	Nitrobacter sp.		*sqhC* [23], *hpnP* [24]	
	Nitrobacter wingoradskyi		*sqhC* [23], *hpnP* [24]	
	Oligotropha carboxidovorans		*hpnP* [24]	
	Rhodoblastus acidophilus	Ic [10]		
	Rhodopseudomonas palustris	Ic + IIf [11, 12]	*sqhC* [23], *hpnP* [24]	
	unaffiliated *Bradyrhizobiaceae*			R_t
Hypomicrobiaceae	*Rhodpmicorbium vannielii*	Ic + Id [11]		
Methylobacteriaceae	*Methylobacterium chloromethanicum*		*hpnP* [24]	
	Methylobacterium extorquens		*hpnP* [24]	
	Methylobacterium nodulans		*hpnP* [24]	
	Methylobacterium populi		*hpnP* [24]	
	Methylobacterium radiotolerans		*hpnP* [24]	
	Methylobacterium silvestris		*hpnP* [24]	
	Methylobacterium sp.		*hpnP* [24]	
Methylocystaceae	*Methylocystis sp.*		*hpnP* [26]	
Rhizobiaceae	*Phyllobacterium spp.*			R_t
	Rhizobium sp.		*sqhC* [23], *hpnP* [24]	
Rhizobiales	*Pedomicrobium* sp.			R_t
	Nordella sp.			
	unaffiliated Rhizobiales			R_t
Rhodobacteraceae	*Rhodovulum sp.*		*hpnP* [26]	
Rhodospirillaceae	*Azospirillum*			R_t, C_t
	Magnetospirillum magnetotacticum		*sqhC* [23, 25]	
	Magnetospirillum magneticum		*sqhC* [23, 25]	
	Rhodospirillum rubrum		*sqhC* [23, 25]	
unaffiliated Rhodospirillaceae				R_t, C_t
Sphingomonadaceae	*Sphingomonas sp.*			R_t, C_t
	Zymomonas mobilis	If +Ig +Ij [12]	*sqhC* [23, 25]	
	Zymomonas mobilis subsp. Mobilis		*sqhC* [23]	
unaffiliated				R_t, C_t
Betaproteobacteria				
Nitrosomonadaceae	*Nitrosomonas europaea*	Ic + Ih [14]	*sqhC* [23, 25]	
	Nitrosomonas eutropha		*sqhC* [23, 25]	
	Nitrosospira multiformis Surinam		*sqhC* [24]	

Comamonadaceae	*Polaromonas* sp.			R_t, C_t
Gallionellaceae	*Gallionella* sp.			C_t
Lysobacteraceae	*Lysobacter* sp.			R_t
unaffiliated				R_t
Gammaproteobacteria				
Methylococcaceae	*Methylobacter psychrophilus*			R_t
	Methylocaldum szegediense	Ib [15]		
	Methylocaldum tepidum	Ia + Ib [16]		
	Methylococcus capsulatus	Ia + Ib [15]	*sqhC* [23, 25]	
	Methylococcus luteus	Ia + Ib [17]		
	Methylomonas methanica	Ia + Ib [15]		
	Methylomonas sp.	Ia + Ib [18]		
	Methylomonas-like strain M5	Ia + Ib [8]		
	Methylosinus trichosporium	Ia + Ih [19]		
	Methylosinus-like strain 29	Ia +Ih [8]		
	Methylovulum-like strain M200	Ia + Ib + Ih [8]		
Deltaproteobacteria				
Desulfovibrionaceae	*Desulfovibrio* sp.	Ia + Ig + Ih [20]		
Geobacteraceae	*Geobacter bremensis*			R_b, C_t, C_b
	Geobacter metallireducens	If+Ig+Ij [21]	*sqhC* [23, 25]	
	Geobacter propionicus			R_b, C_t, C_b
	Geobacter sulfurreducens	If+Ig+Ij [21]	*sqhC* [23, 25]	
	Geobacter uraniumreducens		*sqhC* [23, 25]	
Syntrophaceae	*Syntrophobacter fumaroxidans*		*sqhC* [23, 25]	
	Syntrophus sp.			R_t, R_b, C_t, C_b
Haliangiaceae	*Haliangium*			R_t
unaffiliated				R_t, R_b, C_t, C_b
Thermomicrobia				
unaffiliated				R_t, R_b, C_t, C_b
unaffilitaed Chlorobi				R_t, R_b, C_t, C_b
Verrucomicrobia				
Methylacidiphilacaea	*Methylacidiphilum fumariolicum SolV*	Ia [8]		
Opitutaceae	*Opitutus sp.*			R_t
Verrucomicrobiaceae	*Prosthecobacter sp.*			R_t, C_t, C_b
	unaffiliated			R_t, R_b
NC10 phylum	*Methylomirabilis* sp.	If + IIf [22]		

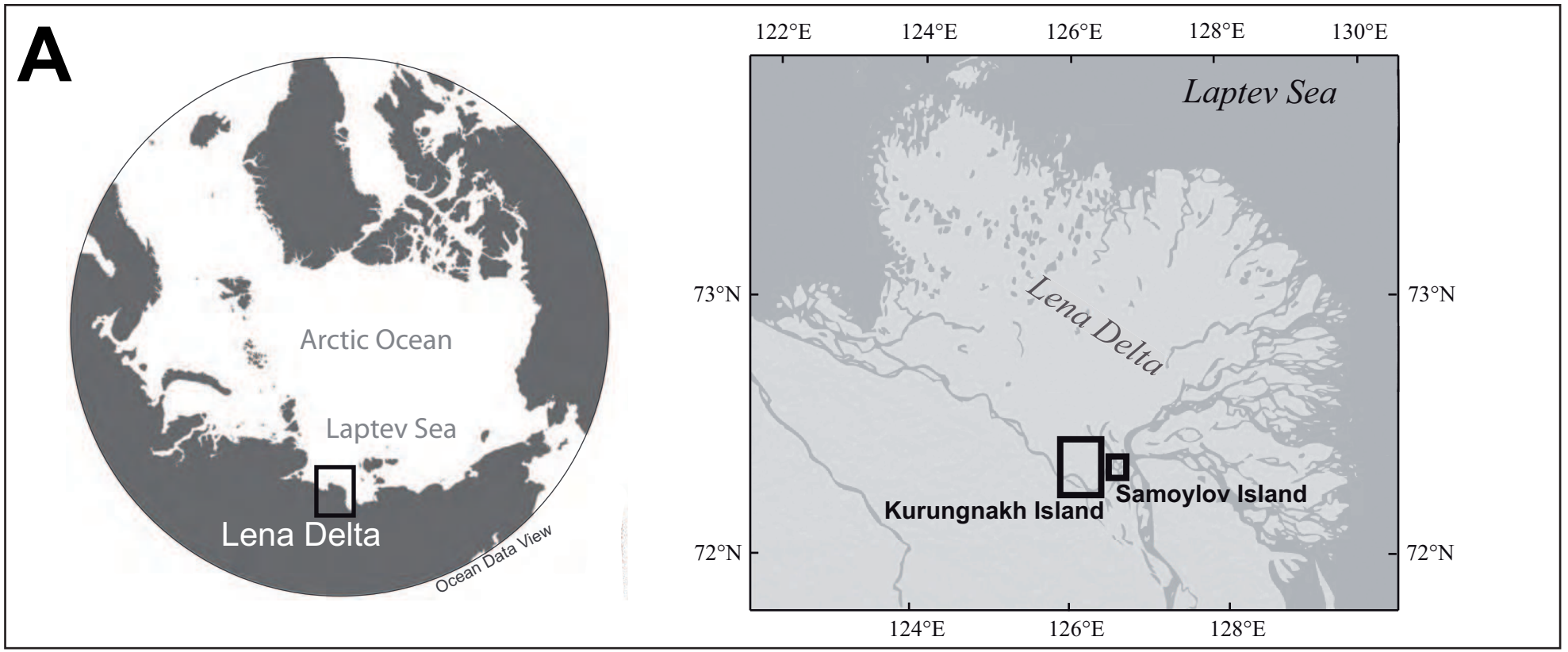
1 - Rosa-Putra et al., 2001, 2 - Talbot et al., 2008, 3 - Bisseret et al., 1985, 4 - Doughty et al., 2009, 5 - Zhao et al., 1996, 6 - Rush et al., 2014, 7 - Vilcheze et al., 1994, 8 - van Winden et al., 2012 , 9 - Bravo et al., 2001, 10 - Neunlist and Rohmer, 1985a, 11 - Talbot et al., 2007, 12 - Flesch and Rohmer, 1989 13 - Rashby et al., 2007, 14 - Seemann et al., 1999, 15 - Coolen et al., 2008, 16 - Neunlist and Rohmer, 1985b, 17 - Cvejic et al., 2000, 18 - Zhou et al., 1991, 19 - Neunlist and Rohmer, 1985c, 20 - Blumenberg et al., 2006, 21 - Eickhoff et al., 2013, 22 - Kool et al., 2014, 23 - Pearson and Rusch, 2009, 24 - Welander et al., 2010, 25 - Fischer and Pearson, 2007, 26 - according to Ricci et al., 2014, 27 - Liebner, 2007, 28 - Liebner et al., 2008;
R_t - Rim top soil (6-10 cm), R_b - Rim bottom soil (28-32 cm), C_t – Centre top soil (6-8 cm), C_b - Centre bottom soil (24-26 cm)

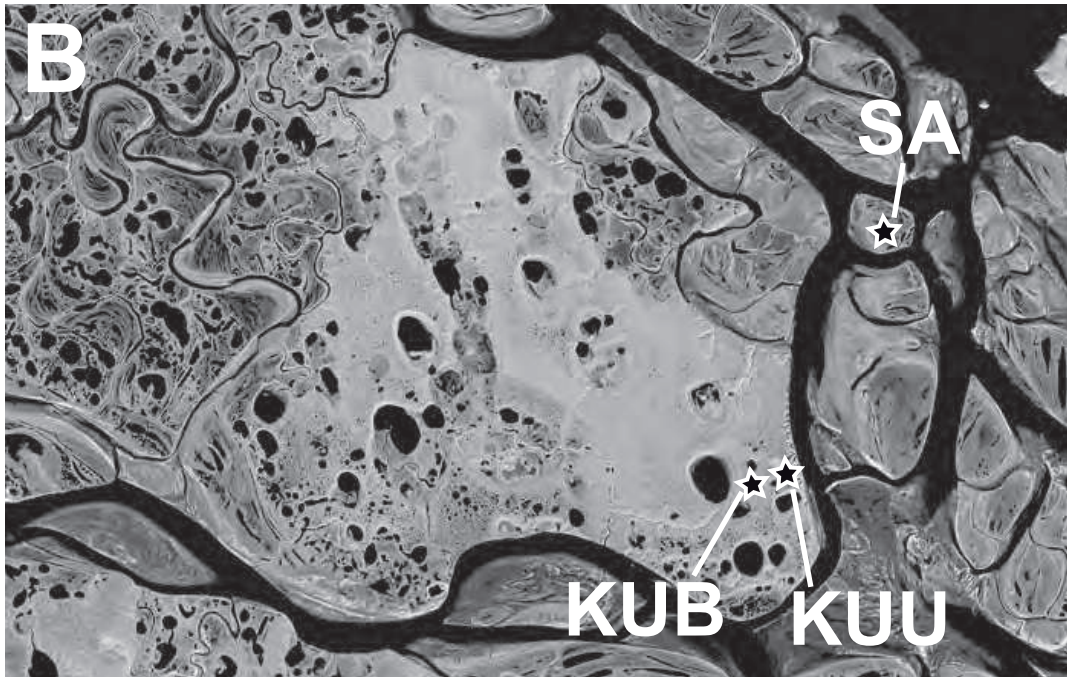
Fig. 1: (A) Study area in the Lena Delta including (B) sampling sites on Samoylov (SA) and Thermokarst basin (KUB) and Upland (KUU) on Kurungnakh Island (Landsat 7 image modified from http:\\www.earthobservatory.nasa.gov/IOTD/view.php?id=2704), and (C) the different landscape structures (polygon centre and rim on SA).

Fig 2: Total BHP concentrations [µg g^{-1} TOC] and structural diversity (total number of BHPs). A) Thermokarst basin (KUB), B) Upland (KUU), C) Samoylov Island (SA). Error bars are the mean error of all BHPs based on the mean SD (15.6%) calculated using relative SD of triplicate injections (5.1% - 42.3%).

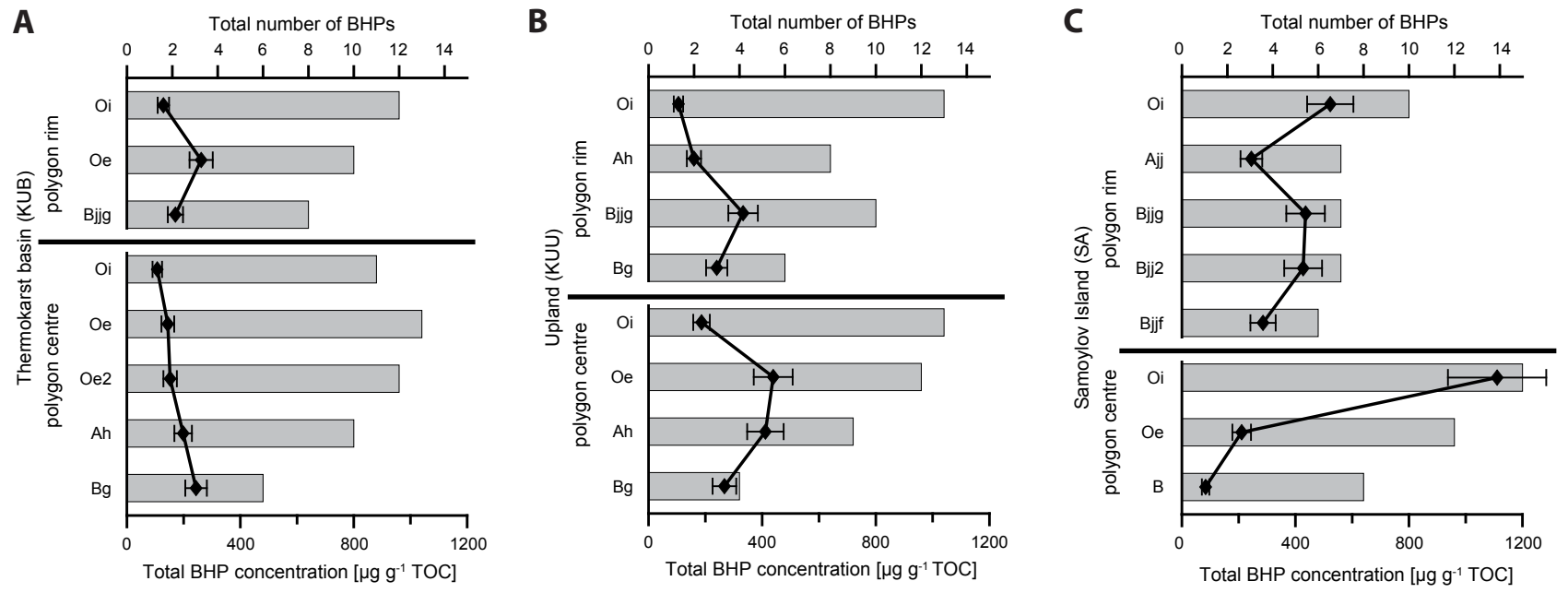
Fig. 3: Relative abundances of BHPs [%] on Kurungnagkh Island in (A) the Thermokarst basin (KUB) and (B) the upland site (KUU), and (C) on Samoylov Island (SA)

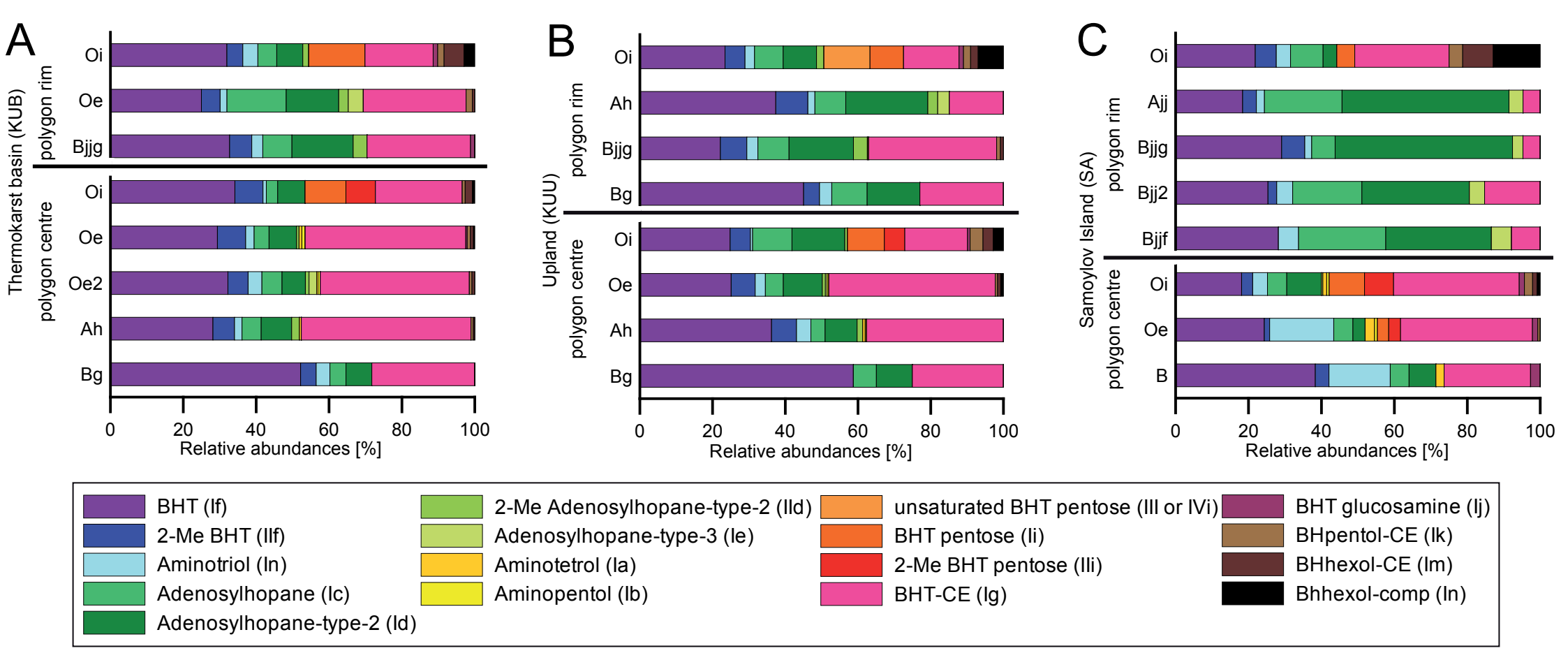
Fig. 4: PCA biplot for BHP data and soil parameters (C content (%), N content (%), water saturation (%), texture (grain size, %), and pH values). Adhp – Adenosylhopane; Adhp-t-2 – Adenosylhopane-type-2; Adhp-t-3 – Adenosylhopane-type-3; 2-Me Adhp-t-2 – 2-Me Adenosylhopane-type-2

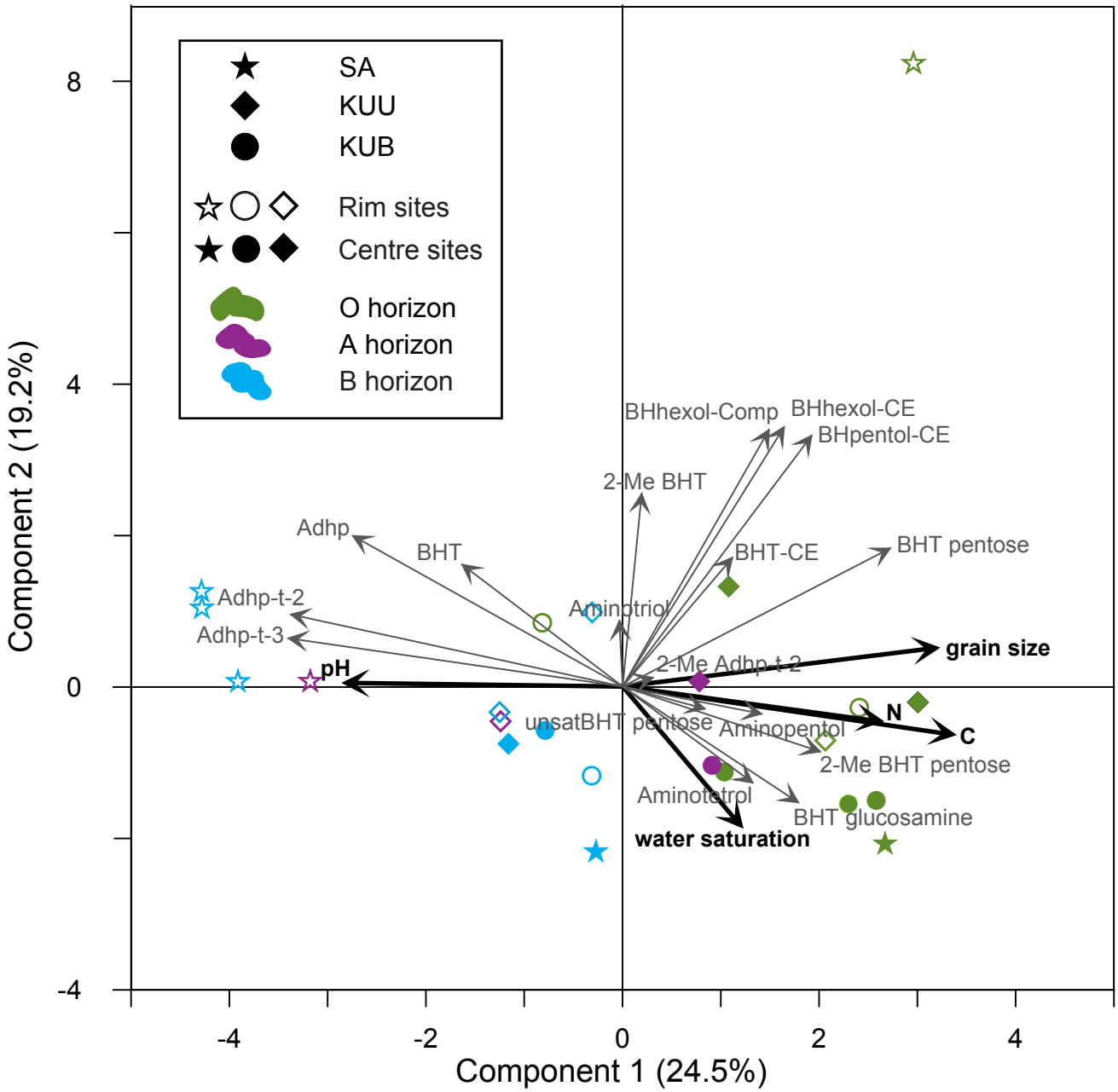

A
Arctic Ocean
Laptev Sea
Lena Delta
Ocean Data View
122°E
124°E
126°E
128°E
130°E
Laptev Sea
Lena Delta
73°N
72°N
Kurungnakh Island
Samoylov Island


B
SA
KUB
KUU


C
Rim
Centre


A
Total number of BHPs
0 2 4 6 8 10 12 14
Thermokarst basin (KUB)
polygon rim
Oi
Oe
Bjjg
polygon centre
Oi
Oe
Oe2
Ah
Bg
0 400 800 1200
Total BHP concentration [µg g-1 TOC]
B
Total number of BHPs
0 2 4 6 8 10 12 14
Upland (KUU)
polygon rim
Oi
Ah
Bjjg
Bg
polygon centre
Oi
Oe
Ah
Bg
0 400 800 1200
Total BHP concentration [µg g-1 TOC]
C
Total number of BHPs
0 2 4 6 8 10 12 14
Samoylov Island (SA)
polygon rim
Oi
Ajj
Bjjg
Bjj2
Bjjf
polygon centre
Oi
Oe
B
0 400 800 1200
Total BHP concentration [µg g-1 TOC]


A
Thermokarst basin (KUB)
polygon rim
Oi
Oe
Bjjg
polygon centre
Oi
Oe
Oe2
Ah
Bg
0
20
40
60
80
100
Relative abundances [%]
B
Upland (KUU)
polygon rim
Oi
Ah
Bjjg
Bg
polygon centre
Oi
Oe
Ah
Bg
0
20
40
60
80
100
Relative abundances [%]
C
Samoylov Island (SA)
polygon rim
Oi
Ajj
Bjjg
Bjj2
Bjjf
polygon centre
Oi
Oe
B
0
20
40
60
80
100
Relative abundances [%]
BHT (If)
2-Me BHT (IIf)
Aminotriol (In)
Adenosylhopane (Ic)
Adenosylhopane-type-2 (Id)
2-Me Adenosylhopane-type-2 (IId)
Adenosylhopane-type-3 (Ie)
Aminotetrol (Ia)
Aminopentol (Ib)
unsaturated BHT pentose (III or IVi)
BHT pentose (Ii)
2-Me BHT pentose (IIi)
BHT-CE (Ig)
BHT glucosamine (Ij)
BHpentol-CE (Ik)
BHhexol-CE (Im)
Bhhexol-comp (In)


SA
KUU
KUB
Rim sites
Centre sites
O horizon
A horizon
B horizon
BHhexol-Comp
BHhexol-CE
BHpentol-CE
2-Me BHT
Adhp
BHT
BHT-CE
BHT pentose
Adhp-t-2
Adhp-t-3
Aminotriol
grain size
pH
2-Me Adhp-t-2
N
C
unsatBHT pentose
Aminopentol
2-Me BHT pentose
Aminotetrol
BHT glucosamine
water saturation
Component 2 (19.2%)
Component 1 (24.5%)
8
4
0
-4
-4
-2
0
2
4